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14. ABSTRACT

Background: Atypical antipsychotics (AAP) are prescribed to numerous autistic patients to treat symptoms of agitation, stereotypic behavior, temper tantrums and self-injury. Despite their ability to ameliorate many behavioral problems, AAP have serious metabolic side-effects which include weight gain, insulin resistance, and increased risk of diabetes and cardiovascular disease. The main therapeutic targets of AAP are the dopamine (DAR) and serotonin (5-HTR) receptors. The general consensus is that AAP cause metabolic disturbances by an exclusive action on the brain. Preliminary Data: We discovered functional DAR and 5-HTR subtypes in human adipose tissue and found that incubation of adipose explants and adipocytes with olanzapine, risperidone and ziprasidone suppressed leptin and adiponectin and alter inteleukin-6 (IL-6) release. Oral delivery of olanzapine to female rats caused a rapid and robust suppression of leptin, a satiety hormone, concomitant with increased food intake and weight gain. Hypothesis and Objectives: We hypothesized that activation of DAR and/or 5-HTR subtypes in adipose tissue contributes to the metabolic side-effects caused by AAP. The overall objective was to establish adipose tissue as a critical target of AAP and elucidate some of the mechanisms by which the drugs alter adipose tissue functions leading to weight gain and the metabolic syndrome.

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Introduction

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Preliminary Data: We discovered functional DAR and 5-HTR subtypes in human adipose tissue and found that incubation of adipose explants and adipocytes with olanzapine, risperidone and ziprasidone suppressed leptin and adiponectin and alter inteleukin-6 (IL-6) release. Oral delivery of olanzapine to female rats caused a rapid and robust suppression of leptin, a satiety hormone, concomitant with increased food intake and weight gain.

Hypothesis and Objectives: We hypothesized that activation of DAR and/or 5-HTR subtypes in adipose tissue contributes to the metabolic side-effects caused by AAP. The overall objective was to establish adipose tissue as a critical target of AAP and elucidate some of the mechanisms by which the drugs alter adipose tissue functions leading to weight gain and the metabolic syndrome.

Specific Aims:

Specific Aim 1: To determine whether weight-inducing AAP stimulate adipogenesis, enhance lipid accumulation and/or alter expression and release of selected adipokines in human and rat adipocytes *in vitro*.

Specific Aim 2: To examine whether drug-induced leptin suppression is a major drive for increased appetite and weight gain in a rat model.

Body

1. Responsiveness of rat sc and vis fat depots to different ligands and AAP

Although AAP bind primarily to DAR and 5-HTR (1), they also affect histamine receptors (2). In addition, adipose tissue from visceral (vis) and subcutaneous (SC) depots have different properties and are likely to respond differentially to the drugs given a dissimilar distribution of receptors as well as their coupling to signal transduction pathways (3,4). Thus, the first experiment was designed to compare the responsiveness of vis and sc explants from rats to the different ligands and AAPs alone, or in combination. As key endpoints, we selected two key adipokines: leptin and adiponectin (adipo), and two key transcription factors: PPARG, which regulates adipogenesis, and SREBP1, which regulates lipid homeostasis. Periovarian (vis) and sc fat from untreated female rats were cut into explants and incubated with serotonin (5HT, 10 nM), dopamine (DA, 10 nM), histamine (His, 10 nM), or a combination of DA/5HT, and DA/5HT/His, each at 10 nM. In addition, explants were incubated with olanzapine (Olan, 1nM and 10 nM) and ziprasidone (Zipr, 10 nM). After 3 days, expression of the four genes was determined by qPCR.

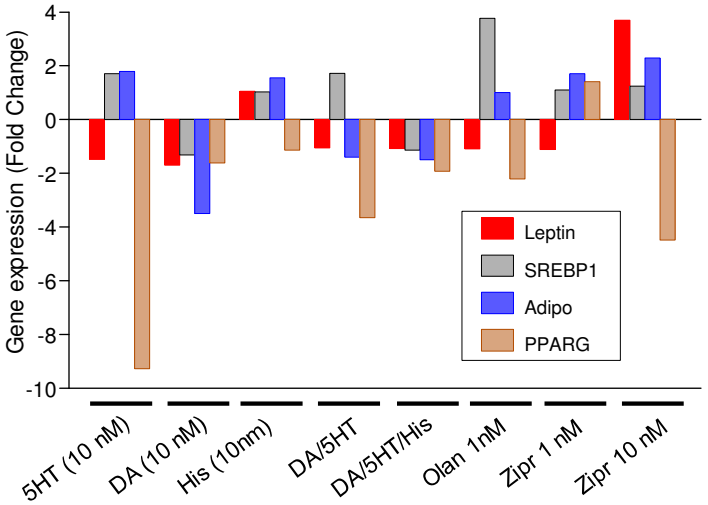

Fig 1: Changes in gene expression in rat sc adipose explants incubated with various compounds.

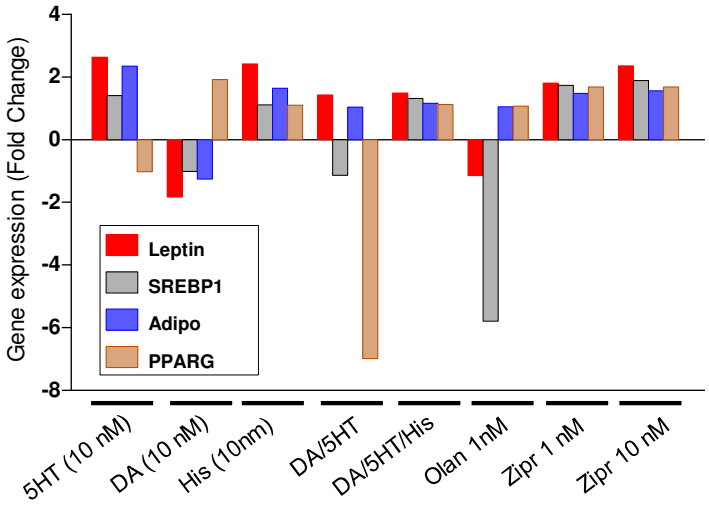

Fig 2: Changes in gene expression in rat vis adipose explants incubated with various compounds.

was slightly stimulated by 5HT, His and Zipr. Although 5HT or DA alone showed no, or slight stimulatory effects on PPARG, their combination showed a marked inhibition. Unlike its stimulatory effect on SREBP1 in sc fat, olanzapine had a significant stimulation in vis fat. Another difference was noted with respect to leptin, whereby 5HT increased leptin expression in vis fat but inhibited its expression in sc fat. The conclusions from these experiments are: 1) there are clearly direct effects of DAR and 5HTR ligands, as well as AAP on both vis and sc fat, 2) histamine receptors do not play a decisive role, 3) a balance between DAR and 5HTR in each depot dictates the overall effects on selected adipose-related genes.

As evident in **Fig 1**, His alone or in combination had little effect on the expression of these genes in sc fat. PPARG was markedly suppressed by 5HT and Zipr, and to a lesser extent by 1 nM Olan or a combination of DA/5HT, while SREBP1 was stimulated by 1 nM Olan. Leptin was increased by Zipr and slightly suppressed by 5HT and DA, while Adipo was slightly stimulated by 5HT and Zipr and significantly suppressed by DA. The combination of DA/5HT did not result in synergism, but in smaller effects.

A rather different profile of gene expression was seen in vis explants (**Fig 2**). PPARG and SREBP1 were markedly suppressed by DA/5HT and 1 nM Olan, respectively, whereas Leptin

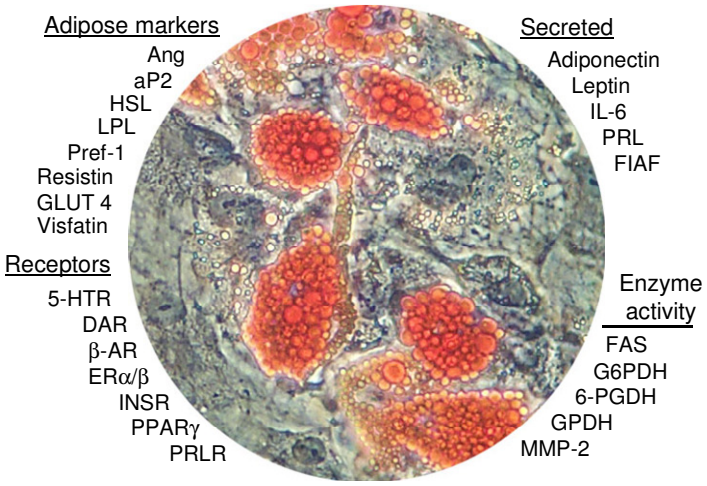

Fig 3: Characterization of LS14 cells. *Left:* expression of adipocyte markers, adipokines and receptors by RT-PCR. *Right:* secreted hormones and enzyme activities.

2. Validation of LS14 cells as representative of vis adipocytes

Although many of our proposed experiments can be done with primary adipocytes, there are several caveats, including limited availability and patient-to-patient variability. On the other hand, the advantage of an adipocyte cell line is homogeneity, availability and ease of genetic/biochemical manipulation. Thus, we opted to carry out full characterization of our LS14 cells, which we have cloned from a patient with metastatic liposarcoma (5). **Fig 3** shows lipid storage in differentiated LS14 cells stained with Oil-red O. We have used multiple methods such as RT-PCR, Western blotting, ELISAs and enzyme assays, to extensively characterize these cells and establish that they resemble vis adipocytes.

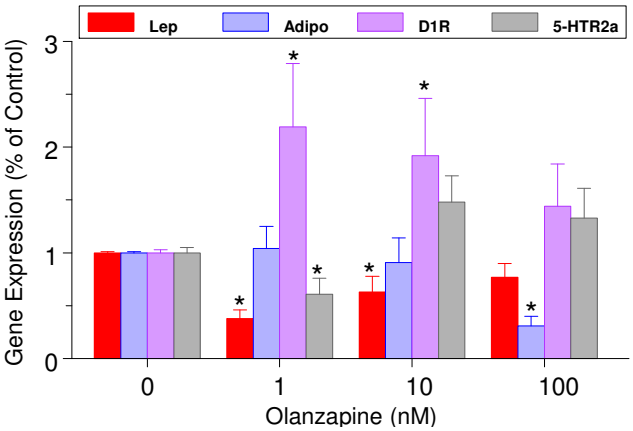

Fig 4: Olanzapine differentially suppresses leptin (Lep), adiponectin (Adipo) and 5-HTR2a, while stimulating D1R expression in LS14 cells, as determined by qPCR.

We next examined whether Olanzapine alter the

expression of leptin, adiponectin, D1R and 5-HTR2a in differentiated LS14 cells. Cells were incubated with increasing doses of olanzapine and ziprasidone for 6 hrs, followed by quantitative PCR. (qPCR). Olanzapine at 1 nM inhibited leptin and 5-HTR2a, but stimulated D1R expression, whereas adiponectin was suppressed only at the high dose (**Fig 4**); ziprasidone was less effective than olanzapine (not shown). Unlike 3T3-L1 adipocytes which downregulate leptin expression, LS14 cells produce significant amounts of leptin. The similar responsiveness of LS14 cells and primary adipocytes to the drugs validated the use of either cell type for studying the metabolic effects of AAP *in vitro*.

Key Research Accomplishments

- Establishing the differential responsiveness of vis and sc fat to various agonists and AAP.
- Demonstrating a direct effect of AAP on the expression of critical adipose-related genes.
- Establishing the resemblance of the LS14 adipocyte cell line to visceral adipocytes and validating its use for these studies.

Reportable Outcome

Presentations in Scientific Meetings:

- Ben-Jonathan: **Antipsychotic-induced Obesity**, BIT's Major Disease Clinical Summit, Warsaw, Poland, November 2013 (Appendix 1)
- Ben-Jonathan: **Antipsychotic induced obesity: Direct actions on the adipocytes**, EuroSciCon, Anti-obesity drug discovery and development, London, April 2014 (Appendix 2).

Conclusion

We are now well positioned to proceed with a a more comprehensive investigation of the effects of AAP on adipogenesis, enhance lipid accumulation and/or alter expression and release of selected adipokines in human and rat adipocytes *in vitro*.

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BIT Congress-Europe

BIT's Major Diseases Clinical Summit-2013

Dr. Nira Ben-Jonathan, Professor, University of Cincinnati, USA

Speech Title: Antipsychotics-Induced Obesity

Speech Session: 4-2

Highlight of Your Speech: (5-6 Points)

- **Atypical antipsychotics induce weight gain and the metabolic syndrome**
- **Adipocytes serve as a major target for the antipsychotics**
- **Drug-induced suppression of leptin results in increased food intake**
- **Suppression of adiponectin contributes to the metabolic syndrome**
- **Human adipocytes can be used to screen for new drugs devoid of metabolic side effects**

Abstract:(within 200 words)

Atypical antipsychotics (AAP) are prescribed to millions of patients with schizophrenia, bipolar disorder, major depression, and autism. Although AAP ameliorate mental dysfunctions, they have serious metabolic side-effects such as weight gain, diabetes, and cardiovascular disease. The primary therapeutic target of the antipsychotics are dopamine (DAR) and serotonin (5-HTR) receptors. The mechanisms underlying the metabolic side effects of AAP have been attributed to their central action. We discovered expression of functional DAR and 5-HTR subtypes in human adipocytes. Incubation of adipose explants and adipocytes with selected AAP suppressed leptin and adiponectin, and increased lipolysis. Treatment of rats with olanzapine caused marked suppression of leptin and adiponectin, and an increase in interleukin-6 (IL-6) expression in fat tissue within 24 hrs, concomitant with increased food intake and weight gain in 2-3 days. We propose that direct activation of DAR and possibly 5-HTR subtypes in adipose tissue by AAP contributes to weight gain and the metabolic syndrome. Human adipocytes could be integrated into the screening paradigm of candidate new drugs for the identification of undesirable metabolic activities prior to costly animal studies and clinical trials. The long term goal is to provide safer drugs to patients requiring treatment with such medications.

Biography:(within 150 words)

Nira Ben-Jonathan, Ph.D, is Professor of Cancer and Cell Biology at the University of Cincinnati, Ohio, USA. She published over 160 manuscripts, edited one book, and contributed 12 chapters to textbooks and encyclopedias. Early in her career she conducted research on the neuroendocrine regulation of pituitary functions. More recently, the focus of her research has shifted to breast cancer and human obesity. Throughout her career, she mentored 65 students, postdoctoral fellows, research scientists and assistant professors. She served on may journal editorial boards and committees of scientific societies. She was awarded the NIH Research Career Development Award, was elected Fellow of the AAAS, was elected Chairman of the Gordon Research Conference, and received the prestigious Rieveschl Award for Outstanding Scientific Research. She has been a member and chairman on numerous study sections of the NIH, the Komen foundation and the DOD.

EuroSciCon Speaker's Form

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Title of the meeting you are speaking at	Anti-obesity drug discovery and development
Title (Mrs, Ms, Miss, Mr, Dr, Other)dx	Dr.
First Name and Surname	Nira Ben-Jonathan
Job Title	Professor
Affiliation (Company/Institution/Hospital/University)	University of Cincinnati
Do you have a website that we can refer to on our site? What is the url?	No
*** Talk Title**	Antipsychotics induced obesity: Direct actions on the adipocytes
*** Brief Abstract of your Talk** (100 words maximum). This will be place on the meeting agenda.	Atypical antipsychotics (AAP) are prescribed to millions of patients with mental diseases. Although AAP ameliorate mental dysfunctions, they have serious metabolic side-effects such as weight gain, diabetes, and cardiovascular disease. We discovered expression of functional dopamine and serotonin receptors in human adipocytes and found that AAP altered many of their functions. We propose that direct actions of AAP on adipose tissue contribute to weight gain and the metabolic syndrome. Human adipocytes could be integrated into the screening paradigm of candidate new drugs for the identification of undesirable metabolic activities prior to costly animal studies and clinical trials.
Up to 5 keywords relevant to your talk	adipocytes, metabolic syndrome, antipsychotics, gene expression, adipokines
Short Professional Biography (100 words maximum) This will be used for promotional purposes and may be used in any press releases about this event .It will also be placed on the meetings web site, the agenda and may be published in our meeting report. Please could you write this in the 3rd person	Nira Ben-Jonathan, Ph.D, is Professor of Cancer and Cell Biology, University of Cincinnati, Ohio, USA. She published 160 manuscripts, edited one book, and contributed 12 chapters to textbooks and encyclopedias. The focus of her research is on the regulation of pituitary functions, breast cancer and human obesity. She mentored 65 students and scientists, served on journal editorial boards and scientific committees, and has been a member and chairman on NIH, DOD and Komen study sections.

	She was elected Fellow of the AAAS and Chairman of the Gordon Research Conference, and received the Rieveschl Award for Outstanding Research.

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